

(19)



Europäisches Patentamt

European Patent Office

Office européen des brevets



(11)

EP 1 005 862 A1

(12)

EUROPEAN PATENT APPLICATION

published in accordance with Art. 158(3) EPC

(43) Date of publication:

07.06.2000 Bulletin 2000/23

(51) Int. Cl.⁷: **A61K 31/35**

// C07D311:62

(21) Application number: 99913707.8

(86) International application number:

PCT/JP99/02063

(22) Date of filing: 19.04.1999

(87) International publication number:

WO 99/53916 (28.10.1999 Gazette 1999/43)

(84) Designated Contracting States:

DE GB

(30) Priority: 21.04.1998 JP 12539298

(71) Applicant:

Mitsui Norin Co., Ltd.

Tokyo 103-8331 (JP)

(72) Inventors:

• YAMAZAKI, Tsutomu
Nerima-ku Tokyo 179-0081 (JP)

• HARA, Yukihiro
Fujieda-shi Shizuoka 426-0078 (JP)

(74) Representative:

VOSSIUS & PARTNER

Siebertstrasse 4

81675 München (DE)

(54) **ANTI-CHLAMYDIA AGENTS**

(57) Anti-Chlamydia agents characterized by containing tea polyphenol; and a method for preventing or treating Chlamydia infection characterized by administering a composition containing a therapeutically efficacious amount of tea polyphenol to the affected part of a patient. The tea polyphenol to be used herein is a component of tea which is a natural product having been widely taken as a drink from ancient times. Thus, tea suffers no problem in safety. Therefore, the tea polyphenol also has no harmful side effect on the human body and is free from any fear of the appearance of a resistant strain.

EP 1 005 862 A1

Description**TECHNICAL FIELD**

5 [0001] The present invention relates to an anti-Chlamydia agent characterized by containing tea polyphenol.

BACKGROUND ART

10 [0002] Bacteria belonging to genus Chlamydia are of a spherical or ellipsoidal shape of 0.2 to 1.5 μm and are unique bacteria which are obligate parasites in cells of eucaryotes.

[0003] Infectious body, which is an ecotype outside host cells, enters into the vacuoles of host cells and converted therein into particles called reticular structural body due to phagocytosis. The reticular formation increases in number by division and matures into an infectious body in a later stage of infection.

15 [0004] Chlamydia are known as pathogens for trachoma and inclusion conjunctivitis (Chlamydia trachomatis), for parrot disease (C. psittaci), as well as known as pathogens for lung fever (pneumonia), sore throat (pharyngitis), bronchitis, sinusitis, otitis media (C. pneumoniae) or others in child. C. trachomatis is a major pathogenic microorganism for sexually transmitted diseases prevailing worldwide.

[0005] For the therapy of Chlamydia infectious diseases, in particular C. trachomatis infectious diseases, oral administration of antibiotics is usually used. In this case, the antibiotics which can be used include doxycycline and mynocyline of tetracycline group, clarithromycin of macrolide group, ofloxacin, tosufloxacin, and sparflaxacin of neu-
20 quinolone group, etc. For the therapy, oral administration is usually continued for about 2 weeks. However, in the case of therapy with antibiotics, the problem of side effects is associated. Hence, drugs of tetracycline group and new quinolone group cannot be administered to pregnant women. Moreover, administration of antibiotics is always accompanied by the risk of the emergence of drug-resistant strains.

25 [0006] Accordingly, an object of the present invention is to find an anti-Chlamydia agent which has no side effect nor the fear of emergence of resistant strains out of natural substances and to provide it.

[0007] The inventors of the present invention have made intensive investigation in order to achieve the above object and as a result they have found that tea polyphenol contained in tea leaves has a remarkable proliferation inhibiting activity against bacteria belonging to genus Chlamydia, thus completing the present invention based on this finding.

30 [0008] Tea used in the present invention has been used as beverage from old times and has no problem on its safety.

DISCLOSURE OF THE INVENTION

35 [0009] The present invention provides an anti-Chlamydia agent containing tea polyphenol.

[0010] Further, the present invention provides a method for treating a Chlamydia infectious disease comprising administering a composition containing tea polyphenol in an amount effective for the therapy of a Chlamydia infectious disease on an affected part of a patient.

BEST MODE FOR CARRYING OUT THE INVENTION

[0011] In the present invention, tea means leaf, stem, xylem, root, and seed obtained from tea tree (*Camellia sinensis*) or mixtures of these. Usually, tea leaves for beverages are generally used as a raw material.

45 [0012] Tea leaves for beverages include various kinds depending on the method for their production, for example, fermented tea such as black tea and paoar tea, semi-fermented tea such as oolong tea and paochong tea, and, non-fermented tea such as green tea, and mixtures of these. In the present invention, any of them may be used.

[0013] Tea polyphenol which can be used in the present invention include tea itself containing said tea polyphenol, extracts from the above tea with water, hot water, organic solvents, hydrous organic solvents, etc., or mixtures thereof. Further, there can be used high tea polyphenol content preparations obtained by purifying tea extracts to a desired
50 degree by organic solvent fractionation or chromatography using adsorbing resins. These methods are described in Japanese Patent Publication Nos. Hei 1-44234, Hei 2-12474, and Hei 2-22755, Japanese Patent Kokai Nos. Hei 4-20589, Hei 5-260907, and Hei 8-109178, etc.

[0014] Tea polyphenol contained in the tea extracts and high tea polyphenol content preparations thus obtained specifically includes catechins, that is, (+)-catechin, (-)-catechin, (+)-gallocatechin, (+)-epigallocatechin, (+)-gallocatechin gallate, (+)-epigallocatechin gallate, (-)-epicatechin, (-)-epicatechin gallate, (-)-catechin gallate, (-)-epigallocatechin, (-)-gallocatechin, (-)-epigallocatechin gallate, (-)-gallocatechin gallate, etc., and teaflavins, that is, teaflavin monogallate A, teaflavin monogallate B, teaflavin digallate, free teaflavin, etc. They are used singly or in combination.

[0015] The above tea polyphenol may be commercially available products, for example, those which contain cate-

chins as a major ingredient, such as trade name: Polyphenon 60 (manufactured by Mitsui Norin Co., Ltd., tea polyphenol content: 60% or more), trade name: Polyphenon 30 (manufactured by Mitsui Norin Co., Ltd., tea polyphenol content: 30% or more), trade name: Polyphenon 70S (manufactured by Mitsui Norin Co., Ltd., tea polyphenol content: 70% or more), trade name: Polyphenon E (manufactured by Mitsui Norin Co., Ltd., tea polyphenol content: 80% or more), etc.
 5 Those which contain teaflavins as a major ingredient include trade name: Polyphenon TF (manufactured by Mitsui Norin Co., Ltd., composition: 16.8% teaflavin, 19.5% teaflavin monogallate A, 16.1% teaflavin monogallate B, 31.4% teaflavin digallate), etc.

[0016] The anti-Chlamydia agent of the present invention can be applied to bacteria belonging to genus Chlamydia such as C. pneumoniae, C. psittaci, and C. pecorum, as well as to C. trachomatis.

10 [0017] In the present invention, upon using the above tea polyphenol, it is combined with a suitable solubilizing agent, suspending agent, base material, or the like and used as a composition in the form of cream, paste, gel, milky lotion, liquid, etc. For example, tea polyphenol and/or tea polyphenol-containing material may be dissolved and/or suspended in purified water, physiological saline, hydrous ethanol etc., and sprayed and/or coated on the affected part such as mucous membrane of respiratory tract, etc. For trachoma, inclusion conjunctivitis, etc., tea polyphenol may be
 15 dissolved in purified water, buffer solution or the like and used as eyedroppers or may be mixed with a base material for ointment and used as eyepaste. Also, it is possible to mix tea polyphenol with cream, paste, gel, ointment, etc. and to coat the affected part such as epithelium of cervical canal, etc. with it.

[0018] The bases for cream, paste, gel, and ointment in the present invention include, for example, hydrocarbons such as white vaseline, yellow vaseline, paraffin, liquid paraffin, squalane, and ceresine; higher fatty acids such as lauric acid, stearic acid, myristic acid, palmitic acid, oleic acid, and linolic acid; higher fatty acid alcohol such as stearyl alcohol, oleyl alcohol, lauryl alcohol, cetyl alcohol, and lanolin alcohol; fatty acid esters such as sorbitan sesquioleate, isopropyl myristate, isopropyl palmitate, and glycerin monostearate; waxes such as beeswax, white beeswax, and lanolin; oils and fats such as avocado oil, olive oil, cacao oil, sesame oil, soy bean oil, castor oil, macadamia nut oil, mink oil, yolk oil, beef tallow, and lard; high molecular compounds such as gum arabi, tragacanth gum, guar gum, karaya gum, dextrin, gelatin, carrageenan, shellac, rosin, casein, sodium carboxymethylcellulose, methylcellulose, ethylcellulose, sodium alginate, nitrocellulose, polyvinyl alcohol, polyvinylpyrrolidone, sodium polyacrylate, polyvinyl methyl ether, laurumacrogol, polyamide resins, and silicone oil, and one or more of these may be selected appropriately and used.

25 [0019] To the above preparations can be added are humectants such as glycerin, propylene glycol, 1,3-butylene glycol, polyethylene glycol, sodium dl-pyrrolidonecarboxylate, sodium lactate, sorbitol, sodium hyaluronate; inorganic substances such as bentonite, kaolin, zinc oxide, and titanium oxide; stabilizers such as methyl paraoxybenzoate, ethyl paraoxybenzoate, propyl paraoxybenzoate, and butyl paraoxybenzoate; antiseptics such as benzalkonium chloride, benzethonium chloride, citric acid, sodium citrate, paraoxybenzoic acid, and boric acid; surfactants such as polyoxyethylene-hydrated castor oil, and known percutaneous absorption promoter, etc., if desired.

30 [0020] In these cases, the concentration of tea polyphenol in the drug may vary depending on the symptom and age of patients, site of use, method of use, etc. and is not limited particularly. Usually, when used in the form of liquid, milky lotion, etc., 0.2 to 50 mg/ml, preferably 1.6 to 10 mg/ml. When used in the form of cream, paste, gel, ointment, etc., the concentration of tea polyphenol in the drug is 0.2 to 200 mg/g, preferably 10 to 100 mg/g.

35 [0021] When the anti-Chlamydia agent of the present invention is used, it is desirable to continue the therapy for a certain period of time, e.g., 2 to 4 weeks, taking into consideration the unique growth cycle of Chlamydia and prevention of recurrence. Although the frequency of use of the agent during that time may vary depending on the factors, such as symptom of the patient, site of use, method of use, concentration of tea polyphenol used, etc., it is possible to continue daily use of 1 to 10 times a day.

[0022] The tea polyphenol which is an active ingredient of the anti-Chlamydia agent of the present invention is highly safe so that it can be used for preventive purposes.

45 [0023] Hereafter, the present invention will be described more specifically by examples. However, the present invention is not limited thereto.

Example 1

50 [0024] As test Chlamydia was used C. trachomatis strain. Chlamydia capable of forming 10^4 inclusion bodies was incubated at 37°C for 30 minutes, 60 minutes or 90 minutes in a SPG (sucrose phosphate glutamate) solution containing tea polyphenol of various concentrations (trade name: Polyphenon 70S manufactured by Mitsui Norin Co., Ltd., catechin content: (-)-epigallocatechin 18.3%, (-)-epicatechin 8.6%, (-)-epigallocatechin gallate 35.9%, (-)-epicatechin gallate 11.2%, (-)-gallocatechin gallate 3.5%). As a control, Chlamydia was incubated similarly in SPG solution containing no tea polyphenol.

55 [0025] After completion of the incubation, each solution was inoculated onto HeLa 229 cell cultivated by monolayer culture and adsorbed by centrifugation at 1,500 rpm for 60 minutes. Thereafter, the inoculum was removed and culture medium for Chlamydia (Eagle Minimum Essential Culture Medium containing 1 µg/ml cycloheximide) was added and

cultivation was performed at 37°C for 72 hours. After completion of cultivation, the culture medium was removed and the cells were fixed with methanol and then stained with fluorescein isothiocyanate (FITC)-labeled anti-*C. trachomatis* monoclonal antibody, followed by counting the number of inclusion bodies of

C. trachomatis.

[0026] The results obtained are shown in Table 1. In the table, the number of inclusion bodies in each treatment is indicated as a relative value with respect to the number of inclusion bodies in the control. As will be apparent from the table, in each group the number of *C. trachomatis* inclusion bodies decreased except when incubated for 30 minutes after addition of 0.2 mg/ml of tea polyphenol. In particular, when incubated for 90 minutes, addition of 1.6 mg/ml or more of tea polyphenol completely inhibited the growth of *C. trachomatis*.

Table 1

Incubation time (minute)	Concentration of Tea Polyphenol (mg/ml)						
	0	0.2	0.4	0.8	1.6	3.2	6.4
30	1.00	1.00	0.58	0.34	0.18	0.10	0.05
60	1.00	0.87	0.41	0.18	0.06	0.02	0.02
90	1.00	0.55	0.07	0.03	0	0	0

Example 2

[0027] As test *Chlamydia* was used *C. trachomatis* strain. *Chlamydia* capable of forming 10^4 inclusion bodies was incubated at 37°C for 90 minutes in a SPG solution containing (-)-epicatechin gallate or (-)-epigallocatechin gallate in various concentrations.

[0028] After completion of the incubation, each solution was inoculated onto Hela 229 cell cultivated by monolayer culture and adsorbed by centrifugation at 1,500 rpm for 60 minutes. Thereafter, the inoculum was removed and culture medium for *Chlamydia* (Eagle Minimum Essential Culture Medium containing 1 µg/ml cycloheximide) was added and cultivation was performed at 37°C for 72 hours. After completion of cultivation, the culture medium was removed and the cells were fixed with methanol and then stained with FITC-labeled anti-*C. trachomatis* monoclonal antibody, followed by counting the number of inclusion bodies of *C. trachomatis*.

[0029] As a result, it was found that (-)-epicatechin gallate and (-)-epigallocatechin gallate completely inhibited the growth of *C. trachomatis* in concentrations of 0.8 mg/ml and 1.6 mg/ml, respectively.

Example 3

[0030] Anti-*Chlamydia* agents were prepared in the following formulations. The compositional analysis values of "Polyphenon E" used as tea polyphenol are as follows.

Composition of "Polyphenon E"

[0031]

(-)-Epigallocatechin	12%
(-)-Epicatechin	9%
(-)-Epigallocatechin gallate	53%
(-)-Gallocatechin gallate	6%
(-)-Epicatechin gallate	4%

EP 1 005 862 A1

Prescription Example 1

[0032]

5

10

15

Zinc oxide	200 g
Liquid paraffin	30 g
White beeswax	32.5 g
Sorbitan sesquioleate	13 g
White vaseline .	604.5 g
"Polyphenon E"	120 g
Total amount	1,000 g

Prescription Example 2

20

[0033]

25

30

35

40

White vaseline	400 g
Cetanol	100 g
White beeswax	50 g
Sorbitan sesquioleate	50 g
Lauromacrogol	5 g
Ethyl paraoxybenzoate (or methyl paraoxybenzoate)	1 g
Butyl paraoxybenzoate (or propyl paraoxybenzoate)	1 g
"Polyphenon E"	120 g
Purified, water	273 g
Total amount	1,000 g

Prescription Example 3

[0034]

45

50

55

White vaseline	250 g
Stearyl alcohol	200 g
Propylene glycol	120 g
Polyoxyethylene-hydrated castor oil 60	40 g
Glycerin monostearate	10 g
Methyl paraoxybenzoate	1 g
Propyl paraoxybenzoate	1 g
"Polyphenon E"	120 g

EP 1 005 862 A1

(continued)

Purified water	258 g
Total amount	1,000 g

Prescription Example 4

[0035]

Beeswax	330 g
Vegetable oil	550 g
"Polyphenon E"	120 g
Total amount	1,000 g

Prescription Example 5

[0036]

White beeswax	50 g
Sorbitan sesquioleate	20 g
White vaseline	810 g
"Polyphenon E"	120 g
Total amount	1,000 g

Prescription Example 6

[0037]

Macrogol (polyethylene glycol) 4,000	440 g
Macrogol (polyethylene glycol) 400	440 g
"Polyphenon E"	120 g
Total amount	1,000 g

Prescription Example 7

[0038]

Stearic acid	200 g
Potassium hydroxide	13 g
Glycerin	100 g
Methyl paraoxybenzoate	1 g

EP 1 005 862 A1

(continued)

Propyl paraoxybenzoate	1 g
"Polyphenon E"	120 g
Purified water	565 g
Total amount	1,000 g

Prescription Example 8

[0039]

Stearic acid	150 g
Isopropyl palmitate	20 g
Lanolin	10 g
Sorbitol	56 g
Potassium hydroxide	10 g
Methyl paraoxybenzoate	1 g
Propyl paraoxybenzoate	1 g
"Polyphenon E"	120 g
Purified water	632 g
Total amount	1,000 g

Prescription Example 9

[0040]

Stearic acid	180 g
Liquid paraffin	20 g
Lanolin	5 g
Sorbitan sesquioleate	20 g
Potassium hydroxide	8 g
Sorbitol	35 g
Methyl paraoxybenzoate	1 g
Propyl paraoxybenzoate	1 g
"Polyphenon E"	120 g
Purified water	610 g
Total amount	1,000 g

Prescription Example 10

[0041]

Boric acid	20 mg
Methyl paraoxybenzoate	0.26 mg
Propyl paraoxybenzoate	0.14 mg
"Polyphenon E"	250 mg
Purified water	total amount 1,000 ml

Prescription Example 11

[0042]

Sodium dihydrogen phosphate anhydride	5.6 mg
Sodium hydrogen phosphate anhydride	2.84 mg
Methyl paraoxybenzoate	0.26 mg
Propyl paraoxybenzoate	0.14 mg
"Polyphenon E"	250 mg
Purified water	total amount 1,000 ml

Prescription Example 12

[0043]

Methyl paraoxybenzoate	0.26 mg
Propyl paraoxybenzoate	0.14 mg
"Polyphenon E"	250 mg
Purified water	total amount 1,000 ml

INDUSTRIAL APPLICABILITY

[0044] According to the present invention, a preventive and therapeutic medicine which is effective on Chlamydia infectious diseases and highly safe is provided. This medicine has no side effect nor the risk of emergence of resistant strains unlike the case where antibiotics are administered.

Claims

1. An anti-Chlamydia agent, which comprises containing tea polyphenol.
2. The anti-Chlamydia agent as claimed in claim 1, wherein the tea polyphenol is at least one selected from the group consisting of (+)-catechin, (-)-catechin, (+)-gallocatechin, (+)-epigallocatechin, (+)-gallocatechin gallate, (+)-epigallocatechin gallate, (-)-epicatechin, (-)-epicatechin gallate, (-)-catechin gallate, (-)-epigallocatechin, (-)-gallocatechin, (-)-epigallocatechin gallate, (-)-gallocatechin gallate, teaflavin monogallate A, teaflavin monogallate B,

teaflavin digallate, and free teaflavin.

3. The anti-Chlamydia agent as claimed in claim 1, wherein Chlamydia is Chlamydia trachomatis.

5 4. A preventive or therapeutic method for a Chlamydia infectious disease, characterized by administering a composition containing tea polyphenol in an amount effective for the therapy of a Chlamydia infectious disease on an affected part of a patient.

10 5. The preventive or therapeutic method as claimed in claim 4, wherein the composition is in the form of cream, paste, gel, ointment, milky lotion, solution or suspension.

15 6. The preventive or therapeutic method as claimed in claim 4, wherein the tea polyphenol in the composition is in a concentration of 0.2 to 50 mg/ml when used in the form of liquid, milky lotion, or the like or 0.2 to 200 mg/g when used in the form of cream, paste, gel, ointment or the like.

20

25

30

35

40

45

50

55

INTERNATIONAL SEARCH REPORT

International application No.

PCT/JP99/02063

A. CLASSIFICATION OF SUBJECT MATTER
Int.Cl.⁶ A61K31/35 // C07D311/62

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
Int.Cl.⁶ A61K31/00-35

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category ^a	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	JP, 10-17484, A (K.K. Venture Control), 20 January, 1998 (20. 01. 98) (Family: none)	1-6
A	JONES, R.B. New treatments for Chlamydia trachomatis. Am. J. Obstet. Gynecol., Vol. 164, No. 6 Pt 2, p.1789-1793 (1991)	1-6

☐ Further documents are listed in the continuation of Box C.

☐ See patent family annex.

* Special categories of cited documents:

- * "A" document defining the general state of the art which is not considered to be of particular relevance
- * "E" earlier document but published on or after the international filing date
- * "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- * "O" document referring to an oral disclosure, use, exhibition or other means
- * "P" document published prior to the international filing date but later than the priority date claimed

- * "T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
- * "X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
- * "Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
- * "Z" document member of the same patent family

Date of the actual completion of the international search
8 July, 1999 (08. 07. 99)

Date of mailing of the international search report
21 July, 1999 (21. 07. 99)

Name and mailing address of the ISA/
Japanese Patent Office

Authorized officer

Facsimile No.

Telephone No.

Form PCT/ISA/210 (second sheet) (July 1992)